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Parent and Metabolite Opioid Drug Concentrations in Unintentional Deaths Involving Opioid and Benzodiazepine Combinations*,†,‡

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Abstract

Effects of benzodiazepines on postmortem opioid parent and parent/metabolite blood concentration ratios were determined for fentanyl-, hydrocodone-, methadone-, or oxycodone-related accidental deaths. These opioids are partially metabolized by the CYP3A4 enzyme system, which is also affected by diazepam and alprazolam. Opioid/metabolite combinations examined were as follows: fentanyl/norfentanyl, hydrocodone/dihydrocodeine, methadone/EDDP, and oxycodone/oxymorphone. Parent opioid concentrations were analyzed for 877 deaths. Parent/metabolite concentration ratios were analyzed for 349 deaths, excluding cases with co-intoxicants present known to interfere with opioid elimination. Alprazolam in combination with diazepam significantly decreased median hydrocodone concentrations by 48% ($p = 0.01$) compared to hydrocodone alone. The methadone parent/metabolite concentration ratio was reduced by 35% in the presence of diazepam compared to methadone alone ($p = 0.03$). Benzodiazepines did not statistically significantly affect fentanyl or oxycodone concentrations. Possible factors affecting opioid concentrations and possible toxicity development, including any differential effects on specific opioids, should continue to be explored.

Keywords

forensic science; accidental death; benzodiazepines; opioids; narcotics; forensic toxicology; drug concentration; metabolites; cytochrome P-450 enzyme system; drug-drug interactions

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Drug-related deaths are a growing problem and a serious public health concern (1). The CDC reported 27,153 overdose deaths nationally in 2008, with 73.8% involving one or more prescription drugs (2). Seventy-eight percent of drug poisoning deaths in 2010 were unintentional, and since 2009, more people have died annually from overdoses than from motor vehicle accidents (3). From 1999 to 2010, drug overdose deaths have more than doubled and those involving an opioid more than quadrupled (4).

Opioid-related deaths typically involve multiple drugs, including benzodiazepines, cocaine, and heroin, among others (5). Benzodiazepines in particular have been identified in many deaths involving fentanyl, oxycodone, methadone, and morphine (6–14). Several studies have reported evidence of concomitant benzodiazepine consumption in 11% to 86% of opioid-related deaths (5–7,12,13,15–25).

Opioids have many physiological effects including alterations in body temperature, sedation, respiratory depression, and euphoria. When taken with opioids, benzodiazepines are typically characterized as reinforcer drugs that augment the opioids' rewarding effects (26). Due to the frequency in which benzodiazepines are identified in opioid-associated fatalities, it is important to examine potential drug–drug interactions that might contribute to mortality. Theoretically, pharmacologic drug interactions may occur with opioids and benzodiazepines through additive sedative and respiratory depressant effects (26). Pharmacokinetic interactions may result from a drug's inhibition or induction of, or competition with, an enzyme responsible for metabolizing another drug.

Several opioids involved in fatalities are metabolized by more than one cytochrome P450 (CYP450) enzyme. Fentanyl is metabolized primarily by the CYP3A4 enzyme to a single major inactive metabolite, norfentanyl (27,28). Hydrocodone is mainly metabolized by CYP3A4 to an inactive metabolite, norhydroco-done; CYP2D6 metabolism is a minor pathway yielding a potent metabolite, hydromorphone (29,30). A third metabolite, dihydro-codeine, is formed from hydrocodone but less is known about this metabolic pathway. However, blood dihydrocodeine concentrations might be important to measure in hydrocodone-related deaths because it could represent the more prevalent metabolite (31).

The metabolism of methadone is complex, and there is a lack of consensus on the relative contribution of individual metabolic enzymes to its biotransformation (32). It has been suggested that CYP2B6 is the major enzymatic pathway, with CYP2C19 and CYP3A4 representing equal secondary pathways (32); others have indicated that CYP3A4 and CYP2D6 are the major metabolic pathways (33). Additionally, methadone is available as a racemic mixture and different CYP enzymes are thought to preferentially metabolize each enantiomer (32). The major inactive metabolite formed during methadone metabolism is 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) (32). Oxycodone is metabolized by the CYP3A4 and CYP2D6 enzymes (29,34). The major metabolic pathway is by CYP3A4 to form a less active metabolite, noroxycodone. The minor pathway, catalyzed by the CYP2D6 enzyme, produces a more potent metabolite, oxymorphone. Oxymorphone is most frequently measured by the West Virginia Office of the Chief Medical Examiner as part of oxycodone death investigations.

Alprazolam and diazepam are two benzodiazepines commonly involved in accidental opioid deaths (16). Alprazolam is primarily metabolized by CYP3A4 (35). Diazepam is metabolized by both CYP3A4 and CYP2C19 (35), and it has also been cited as a CYP3A4 inhibitor (36).

In living subjects, sedative effects were observed earlier and for a longer duration when opioids and benzodiazepines were given together as opposed to separately, possibly representing a drug–drug interaction (37). Parent drug to metabolite concentration ratios of opioids have been determined in a few pharmaco-kinetic studies, but the results have not been reproducible. Inhibition of the CYP2D6 metabolic pathway of oxycodone has yielded a higher blood noroxycodone concentration, presumably through greater oxycodone metabolism by CYP3A4 (38,39). One study observed an increase in oxymorphone concentrations (mediated by the CYP2D6 enzyme) when the CYP3A4 pathway was inhibited (39); yet, another study did not observe such an increase in oxymorphone concentrations (40).

A limited number of human studies have examined a potential benzodiazepine and opioid pharmacokinetic interaction. Two small pharmacokinetic studies failed to show either a consistent decrease in methadone renal clearance or increased methadone blood concentrations over time after diazepam consumption (41,42). However, based on postmortem blood concentrations, one case report suggested that oxycodone's metabolism was reduced by concomitant clonazepam intake (8).

In death investigations of potential contributory drug toxicity, medical examiners often measure both parent drug and metabolite concentrations. The parent drug to metabolite ratio can help clarify the manner of death or differentiate an acute drug overdose from chronic drug use, abuse/misuse versus therapeutic drug use, or between ingestion of a parent drug and ingestion of an active metabolite that might also be prescribed. Parent drug to metabolite ratios have been described most often for methadone.

To date, very few studies have examined parent and metabolite postmortem opioid drug concentrations in combined opioidand benzodiazepine-related deaths. One such study measured concentrations of both enantiomers of methadone and its metabolite EDDP. Decedents were divided based on the presence of co-intoxicants, that is, other analgesics, benzodiazepines, and other drugs. There was no statistically significant difference in the ratio of methadone to EDDP concentrations across groups (10). Limitations of this study included a small sample size and inclusion of a number of intentional deaths (10).

West Virginia has one of the highest drug overdose death rates in the United States (3). In 2010, the drug-related death rate was 28.3 per 100,000 population, with a 21.3% increase in these deaths between 2009 and 2010 (43). Of 515 West Virginia drug-related deaths in 2010, 82.8% were considered unintentional. The top five drugs/drug classes identified in these deaths were opioids, benzodiazepines, antidepressants, alcohol, and cocaine (43). The opioids most commonly identified in the West Virginia drug-related deaths (2005–2010) were fentanyl, hydro-codone, methadone, and oxycodone (unpublished data, West Virginia Office of the Chief Medical Examiner). In light of the frequency of combined opioid-and-

benzodiazepine-related deaths and the relative lack of data characterizing the opioid concentrations in such deaths, it is important to examine the potential effects of benzodiazepines on opioid concentrations.

This study examined postmortem parent drug to metabolite concentration ratios of four commonly encountered opioids at least partially metabolized by the cytochrome P450 enzyme system, fentanyl/norfentanyl, hydrocodone/dihydrocodeine, methadone/EDDP, and oxycodone/oxymorphone, in the presence and absence of two commonly identified co-intoxicant benzodiazepines, alprazolam, and diazepam. It is hypothesized that with potential competition with or inhibition of CYP3A4 by alprazolam or diazepam, the opioid parent concentrations and parent to metabolite concentration ratios would be higher compared to the opioid alone. The findings will contribute to better understanding of possible interactions between benzodiazepines and opioids in unintentional drug-related deaths and will assist in the interpretation of parent opioid and metabolite concentrations during opioid-related death investigations.

Materials and Methods

Case Identification

A Forensic Drug Database (FDD) was initially created in 2005 to track and analyze data from all drug-related deaths in West Virginia. Pertinent death investigation data are entered directly into the FDD by the WV Office of the Chief Medical Examiner (OCME). These data include demographic information (age, gender, zip code location of death), condition of the body, body mass index, death certificate data (cause and manner of death, contributory factors to death, the drugs identified as a cause or contributor to the death), the known or suspected route of drug administration, whether the decedent had a prescription for any controlled substances identified (utilizing the West Virginia controlled substances monitoring program database), medical history (available for most decedents), key autopsy findings (all decedents), and toxicological analyses (all drug-related deaths). At the time of this study, the database contained information on 2784 drug-related deaths that occurred from January 2005 through most of 2010 (data entry ongoing).

For the study analyses, all accidental drug-related deaths involving four or less concomitant drugs were identified. Cases were excluded that involved more than four co-intoxicant drugs to minimize the possibility of confounding effects. Of the remaining cases, those in which fentanyl, hydrocodone, methadone, or oxycodone were determined to have caused or contributed to the death were identified. Cases were excluded if more than one opioid was identified by toxicological analyses or if benzodiazepines other than alprazolam or diazepam were present. This resulted in the dataset used for the parent opioid concentration analyses by number of drugs present (Group A). For the parent opioid and parent to metabolite opioid concentration ratio analyses in the presence or absence of alprazolam and diazepam, cases involving contributory co-intoxicants that are inhibitors or inducers of CYP3A4 or CYP2D6 or that have known pharmacokinetic interactions with opioids were also excluded (Group B).

Toxicological Analyses

Study cases were autopsied by the West Virginia Office of the Chief Medical Examiner, and blood (subclavian, femoral, iliac, and/or heart), liver, gastric contents, vitreous fluid, and urine (or aqueous bladder rinse) samples were typically obtained. All were analyzed for volatile compounds, including ethanol by GC-FID with t-butanol as internal standard. Blood or urine was screened by enzyme-multiplied immunoassay technique using reagents purchased from Microgenics-Thermo Scientific (Middletown, VA). The immunoassay test panel included amphetamine/methamphetamine, barbiturates, benzodiazepines, buprenorphine, cocaine metabolite, fentanyl, marijuana metabolite, methadone, opiates, oxycodone/oxymorphone, and tricyclic antidepressants. In preparation for immunoassay, a 1.0 mL aliquot of blood was precipitated using acetone, inverted for 10 min, and centrifuged at 3000 × *g*. The organic layer was then dried at 37°C under a stream of nitrogen gas. The resultant extract was reconstituted using deionized water. Drug screening of protein-precipitated blood extracts was also performed beginning in 2009–2010 using LC/TOF-MS or LC-MS/MS. Confirmation and quantitation of positive screen results were performed using LC-MS or LC-MS/MS with deuterated internal standards or GC/MS with proadifen or barbital as internal standards.

Statistical Analyses

Cochran–Mantel–Haenszel tests were used to compare age distribution, gender, number of concomitant drugs present, and benzodiazepine usage (i.e., neither, alprazolam, diazepam, or both) among the opioid groups. Kruskal–Wallis tests were performed to analyze differences in parent opioid concentrations based upon the presence or absence of benzodiazepines and number of concomitant drugs present and also to compare parent to metabolite concentration ratios for each opioid in the presence and absence of alprazolam and diazepam. For overall statistically significant opioid concentration comparisons, post hoc pairwise Wilcoxon rank-sum tests were performed. The level of significance of each pairwise test was reduced from 0.05 to 0.0083 (0.05/6) using the conservative Bonferroni correction to reduce the likelihood of Type I error with the multiple pairwise comparisons. All of the data analyses were carried out in SAS version 9.3 (SAS Institute, Cary, NC).

Results

Parent Opioid and Drug/Metabolite Concentrations

Of the 2355 accidental drug-related deaths in the FDD, 877 fatalities were included in Group A and 349, all of which had metabolite concentrations, were included in Group B. Figure 1 provides a flow chart for those cases included in the opioid parent drug concentration analyses (Group A) and those cases in the opioid parent and parent to metabolite concentration ratio analyses that excluded deaths in which known opioid drug–drug interactions or alcohol was present (Group B).

Of the single opioid-related deaths involving fentanyl, hydrocodone, methadone, or oxycodone, methadone was most often present, followed by oxycodone, and hydrocodone and fentanyl (Table 1). Most of these deaths occurred in males with an approximate 2:1 ratio between male and female decedents across all four opioid groups. Most decedents were aged

26 to 44 years (constituting from 42% to 56% of decedents) for each opioid present, with 45 to 54 years of age being the next most prevalent group. The age distributions were similar between fentanyl and oxycodone. However, the ages of decedents who ingested hydrocodone were statistically significantly different compared to the other three opioids, with 52.6% of these individuals being 45 years of age and older, compared to 27–40.3% for the decedents ingesting fentanyl, methadone, or oxycodone. Methadone decedents were significantly younger than those ingesting hydrocodone, fentanyl, or oxycodone, with 73% of those who used methadone being 44 years of age or younger, compared to ranges of approximately 47% (hydrocodone) to 67% (fentanyl) for the other opioids in that age range.

Other drugs were frequently identified as contributors to the opioid-related deaths, with a single opioid present in only approximately 9–27% of cases. Hydrocodone was statistically significantly less likely to be present alone compared to the other three opioids. Benzodiazepines (alprazolam and diazepam) were often found in combination with one of the opioids, being present in from approximately 41% (methadone) to 68% (hydrocodone) of the Group A fatalities. The presence of benzodiazepines was statistically significantly different among the opioids, with alprazolam identified in almost double the proportion of hydrocodoneand oxycodone-related deaths as compared to the fentanyl- and methadone-associated fatalities.

In general, as the number of identified drugs increased from 1 or 2 drugs present to 3 or 4 drugs present, the median concentrations of hydrocodone, methadone, and oxycodone statistically significantly decreased (Table 2). The median drug concentrations were similar between cases in which only a single opioid was present and those fatalities in which an opioid and 1 other drug was present. Similar but significantly lower blood opioid concentrations were present when a total of three or four cointoxicants were identified. No statistically significant relationship was found among the median fentanyl blood concentrations and the number of drugs present, with the concentrations similar across categories.

Median opioid parent and parent/metabolite concentration ratios for Group B deaths in the presence and absence of alprazolam and/or diazepam are shown in Table 3. There were no statistically significant differences in the median parent drug or the median parent to metabolite blood concentration ratios for fentanyl or oxycodone regardless of the presence/absence of benzodiazepines. Median fentanyl concentrations in particular were identical regardless of whether one or both benzodiazepines were present or absent. There was a statistically significant difference overall among the median hydrocodone parent drug concentrations in the presence/absence of a benzodiazepine (Table 3). This was statistically significant for hydrocodone alone (0.31 μg/mL) versus hydrocodone with both alprazolam and diazepam present (0.16 μg/mL; pairwise Wilcoxon rank-sum; $p = 0.01$); however, this comparison was nonsignificant ($p = 0.06$) after the conservative Bonferroni correction was applied.

There was no statistically significant difference among the median parent methadone concentrations in the presence or absence of a benzodiazepine (Table 3). There was a significant difference overall among the median methadone parent to metabolite

concentration ratios (Table 3); this was statistically significant for the median ratio with methadone alone (11.85) versus the ratio in the presence of diazepam (7.67; pairwise Wilcoxon rank-sum; $p < 0.04$ with Bonferroni correction).

Discussion

Previous studies have found co-intoxicants to be frequently involved in opioid-related deaths, with benzodiazepines identified in 11–86% of these fatalities (5,6,12,13,15–25). The present analysis similarly found that single opioid fentanyl-, hydroco-done-, methadone-, and oxycodone-related deaths were uncommon, with concomitant be nzodiazepines present in 41% to 68% of these deaths. Hydrocodone-related deaths were most likely and methadone-related deaths were least likely to have at least one benzodiazepine present. Alprazolam was more often identified in the hydrocodone- and oxycodone-associated deaths than in the fentanyl- and methadone-related fatalities.

The 95th percentile opioid concentrations in this study were generally much higher and demonstrated greater variability than the median values, likely indicative of skewed concentration data. Thus, medians were used for statistical comparisons as has been recommended by others (44,45). Previous studies have reported significantly lower oxycodone blood concentrations when multiple drugs were involved in the deaths, compared to single oxycodone-related deaths (44,46), indicating the potential for toxic interactions among these drugs. The present study found significant reductions in median blood concentrations for hydrocodone, methadone, and oxycodone when 3 or 4 drugs were identified compared to when only one or two drugs were involved. Thus, lower opioid concentrations might be needed to achieve the threshold for fatal intoxication in the presence of multiple co-intoxicants. Interestingly, median fentanyl concentrations were not significantly affected by an increasing number of co-intoxicant drugs. The reason for this difference is unknown and should be verified using a larger number of fentanyl cases.

In the presence of benzodiazepines that might inhibit CYP3A4, diminished metabolism of opioids affected by that enzyme might result in higher parent opioid concentrations as well as higher parent/metabolite concentration ratios. The present study did not find statistically significant differences among the median opioid concentrations in the presence or absence of a benzodiazepine, with the exception of an overall significant reduction in hydrocodone concentrations alone compared to hydrocodone concentrations in the presence of both alprazolam and diazepam, although the statistical significance was lost when a multiple comparison correction was used. Deaths in which any co-intoxicant drug was present that was a known inhibitor or inducer of CYP3A4 or CYP2D6, or was otherwise documented to affect the concentrations of the opioids studied, were excluded so that any effect observed would be more likely to result from the benzodiazepines. Certain aspects of hydrocodone metabolism in humans are not fully understood. This adds to the complexity of interpreting parent and metabolite drug concentrations in postmortem cases where several other confounding factors might exist.

Inconsistent effects of benzodiazepines on opioid concentrations have been reported in the literature. Two studies in living subjects (41,42) demonstrated similar results to the present

study, with no effect on methadone concentrations evident in the presence of diazepam. A case series by Rogers et al., found that the postmortem blood methadone concentrations (0.14–0.70 mg/L) in cases involving co-administration of methadone and alprazolam were lower than literature reports for single drug overdoses (0.4–1.8 mg/L and 122–390 μg/L) (47). In contrast, Andreassan et al. studied factors that might influence oxycodone pharmacokinetics and found that use of a CYP3A4 inhibitor (i.e., fluconazole, clarithromycin, verapamil, nelfinavir, itraconazole, and diltiazem) increased oxycodone serum concentrations (48). Although this study does not provide support for a pharmacokinetic interaction between the opioids and benzodiazepines studied, an enhanced pharmacological effect could be responsible for lower opioid concentrations observed when opioids and benzodiazepines are co-ingested. Other studies have speculated that concomitant opioid–psychotropic medication ingestion can lead to an additive pharmacological effect with respiratory depression occurring at lower opioid doses (11,13,19,44,47,49,50).

The drug/metabolite ratio has been proposed as a marker reflecting recent opioid use and the likelihood of a period of abstinence (51). A low metabolite to parent concentration ratio suggests acute consumption. By contrast, when low parent drug/ metabolite concentration ratios are observed, longer term opioid use may be inferred. In this study, the only statistically significant difference seen in the median opioid parent to metabolite concentration ratio in the presence of a benzodiazepine was for the methadone ratio in the presence of diazepam, which was reduced by approximately 35% compared to methadone alone. Methadone/EDDP concentration ratios ranging from 0.1 to 60 have been reported in the literature (10,52,53). Our methadone ratios in the presence and absence of benzodiazepines fell within this range. Buchard et al. measured both R- and S-enantiomers of methadone and its metabolite, EDDP, in 90 postmortem cases and found no evidence that drug–drug interactions influenced the ratio of methadone to metabolite for either enantiomer for comparisons among methadone only, methadone and another strong analgesic, methadone and a benzodiazepine, and methadone and other drugs (10). In our study, chronic use of methadone may have contributed to the lower parent/metabolite concentration ratio observed with diazepam. Alternatively, due to the complex metabolism of methadone, the presence of a benzodiazepine may have minimal pharmacokinetic effects on the opioid.

There were several potential limitations to this study. While efforts were made to be complete and accurate when entering relevant information from each fatality into the FDD, it is possible that some errors were made during data entry. Some data related to drug concentrations were either not obtained for a decedent or not available in their file; these cases, relatively small in number, were excluded. The amount of drug actually taken in most fatalities cannot be accurately established, and the route of administration might not be definitively known. Although we eliminated fatalities with co-intoxicants that were known to be inducers/inhibitors of CYP 3A4 or 2D6 or were documented to affect opioid pharmacokinetics, it is possible that some inhibitors or inducers of CYP metabolism that are not identified during toxicological testing (e.g., St. John's Wart, azole antifungals, grapefruit juice) were present in decedents. A variety of other factors may affect postmortem drug concentrations in drug-related deaths including age, gender, body mass index, organ function, the postmortem interval, and genetic variability (54). Certain CYP enzymes, particularly CYP2D6, show genetic variation which leads to interindividual differences in

therapeutic response and adverse side effects (55). The increased risk of methadone mortality has been associated with the CYP2B6*6 allele (56). Co-administration of methadone and benzodiazepines can be toxic in the presence of gene variation OPRM1A118g (56). Pharmacogenomics testing was not conducted on samples. Given the association between ethnicity and pharmacogenomics, this variable may provide a potential explanation for the differences in the ratios of drug/metabolites. The small sample size for some of the individual groups analyzed, particularly for Group B, could have impacted the study's power. A limitation of postmortem data collection is that some drugs undergo extensive postmortem redistribution. This is of greater concern for highly lipophilic drugs such as methadone, fentanyl, and drugs with large volumes of distribution (31,57). In this study, drug concentration measurements were obtained using blood from the subclavian or femoral vein. As heart blood is most susceptible to artifactual changes after death (22), a small number of overdose deaths were excluded in which peripheral blood was unavailable and heart blood or tissues were used for the toxicological analyses.

Conclusion

Analysis of 877 drug-related deaths found that the presence of 3 or 4 co-intoxicants significantly decreased hydrocodone, methadone, and oxycodone, but not fentanyl, parent drug concentrations compared to when these opioids were present alone or combined with 1 other drug. Median opioid concentrations were not statistically significantly affected by the presence or absence of diazepam or alprazolam, with the possible exception of hydrocodone. The presence of diazepam significantly decreased the methadone parent drug/metabolite concentration ratio compared to methadone alone. Further research examining drug concentrations from larger numbers of accidental opioid-related deaths is warranted to determine if significant differences exist between individual opioids in the presence of specific co-intoxicants. Decedent characteristics or combinations of factors that might affect postmortem opioid concentrations and their potential toxicity should also be explored.

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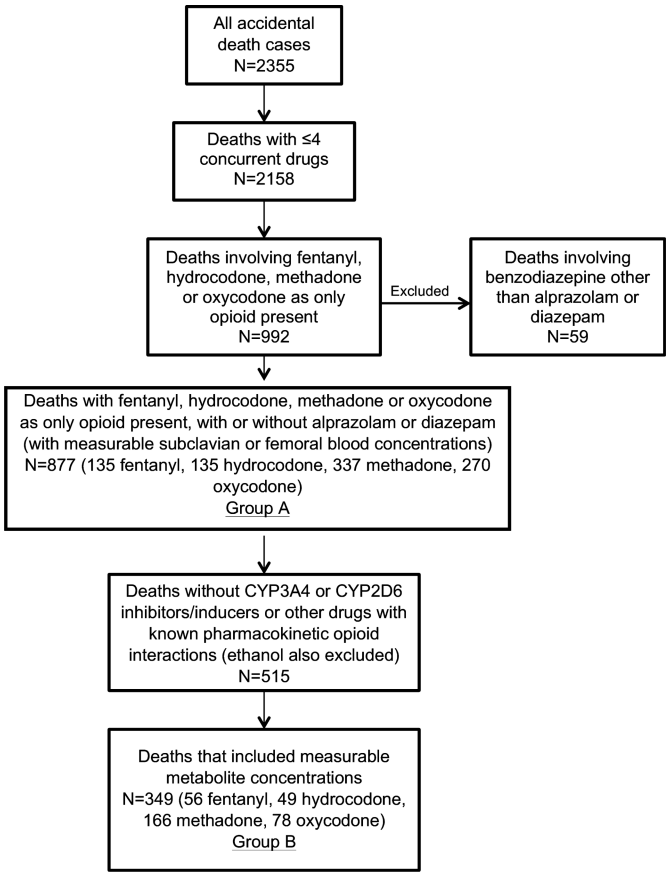

FIG. 1.
Flow chart of decedent cases analyzed.

TABLE 1

Decedent characteristics of cases analyzed (Group A).

Characteristic	Opioid				*p* Value
	Fentanyl (*n* = 135)	Hydrocodone (*n* = 135)	Methadone (*n* = 337)	Oxycodone (*n* = 270)	
Age, *n* (%)					
≤25	15 (11.1)	8 (5.9)	81 (24)	35 (13)	<0.001 *,‡
26–44	75 (55.6)	56 (41.5)	165 (49)	126 (46.7)	
45–54	37 (27.4)	52 (38.5)	80 (23.7)	87 (32.2)	
55–74	8 (5.9)	19 (14.1)	11 (3.3)	22 (8.1)	
Gender, *n* (%)					
Male	86 (63.7)	87 (64.4)	246 (73)	188 (69.6)	0.13
Female	49 (36.3)	48 (35.6)	91 (27)	82 (30.4)	
Benzodiazepine, *n* (%)					
Neither	76 (56.3)	43 (31.9)	200 (59.3)	108 (40)	<0.001 †,‡
Alprazolam	18 (13.3)	47 (34.8)	59 (17.5)	90 (33.3)	
Diazepam	23 (17)	25 (18.5)	65 (19.3)	44 (16.3)	
Both	18 (13.3)	20 (14.8)	13 (3.9)	28 (10.4)	
Number of drugs present, *n* (%)					
1	29 (21.5)	12 (8.9)	92 (27.3)	48 (17.8)	<0.001 *,‡
2	47 (34.8)	52 (38.5)	150 (44.5)	125 (46.3)	
3	40 (29.6)	50 (37.0)	73 (21.7)	73 (27.0)	
4	19 (14.1)	21 (15.6)	22 (6.5)	24 (8.9)	

*Statistically significant; Cochran–Mantel–Haenszel.

†Statistically significant; Chi-square.

‡Pairwise opioid comparisons (Bonferroni correction): Age – All statistically significant ($p < 0.04$) except fentanyl versus oxycodone ($p = 1$). Benzodiazepines – All statistically significant ($p < 0.015$) except hydrocodone versus oxycodone ($p = 1$). Number of drugs present – All statistically significant ($p < 0.03$) except fentanyl versus hydrocodone, oxycodone ($p > 0.2$).

TABLE 2

Parent drug concentrations by number of drugs involved (Group A).

	Parent Drug Concentration		
Opioid	**Median (μg/mL)**	**95th percentile (μg/mL)**	***p*-Value**
Fentanyl			
# drugs present (*n*, %)			
1 (29, 16.3%)	0.015	0.076	0.3135
2 (47, 34.8%)	0.016	0.066	
3 (40, 29.6%)	0.011	0.043	
4 (19, 14.1%)	0.013	0.049	
Hydrocodone			
# drugs present (*n*, %)			
1 (12, 8.9%)	0.27	1.20	0.0002 *
2 (52, 38.5%)	0.20	0.67	
3 (50, 37%)	0.13‡	0.53	
4 (21, 15.6%)	0.12†	0.32	
Methadone			
# drugs present (*n*, %)			
1 (92, 27.2%)	0.42	1.36	0.0073 *
2 (150, 44.5%)	0.42	1.47	
3 (73, 21.7%)	0.38	0.87	
4 (22, 6.5%)	0.32†	0.72	
Oxycodone			
# drugs present (*n*, %)			
1 (48, 17.8%)	0.52	1.62	0.0046 *
2 (125, 46.3%)	0.40	1.45	
3 (73, 27%)	0.27†	0.92	
4 (24, 8.9%)	0.26	1.31	

* Statistically significant differences among median values; Kruskal–Wallis.

† Statistically significant ($p < 0.05$) compared to 1 or 2 drugs; post hoc pairwise Wilcoxon rank-sum.

‡ Statistically significant ($p < 0.05$) compared to 1 drug; post hoc pair-wise Wilcoxon rank-sum.

TABLE 3

Parent drug concentrations and parent/metabolite ratios in presence/absence of alprazolam and diazepam (Group B).

Opioid (*n*) and Presence/Absence of Benzodiazepine[†] (*n*, %)	Parent Drug Concentration			Parent/Metabolite Concentration Ratio		
	Median (μg/mL)	95th Percentile (μg/mL)	*p*-Value[*]	Median	95th Percentile	*p*-Value[*]
Fentanyl (56)						
A (12, 21.4%)	0.01	0.07	0.80	3.64	9.33	0.52
D (5, 8.9%)	0.01	0.03		1.73	13.00	
A + D (11, 19.6%)	0.01	0.03		3.45	7.06	
Neither (28, 50%)	0.01	0.06		4.13	25.27	
Hydrocodone (49)						
A (23, 46.9%)	0.20	0.50	0.02[‡]	4.50	22.5	0.06
D (6, 12.2%)	0.38	1.30		3.01	8.13	
A + D (10, 20.4%)	0.16	0.30		3.17	7.00	
Neither (10, 20.4%)	0.31	1.20		8.11	20.00	
Methadone (166)						
A (31, 18.7%)	0.44	1.44	0.10	8.50	26.00	0.01[‡]
D (35, 21.1%)	0.39	1.17		7.67	23.00	
A + D (6, 3.6%)	0.73	1.04		6.94	12.43	
Neither (94, 56.6%)	0.50	1.50		11.85	48.40	
Oxycodone (78)						
A (26, 33.3%)	0.44	1.50	0.45	7.80	44.00	0.14
D (10, 12.8%)	0.40	1.69		10.48	83.00	
A + D (11, 14.1%)	0.40	1.13		3.53	23.00	
Neither (31, 39.7%)	0.51	1.45		3.91	18.40	

[*] Differences among median values.

[†] A = alprazolam, D = diazepam, A + D = alprazolam and diazepam.

[‡] Statistically significant; Kruskal–Wallis.